XII. *On Variation in the Colour of Cocoons, Pupæ, and Larvæ: further experiments.* By William Bateson, M.A., Fellow of St. John's College, Cambridge. Communicated by Dr. David Sharp, M.A., F.R.S.

[Read October 5th, 1892.]

I. *The colour of the cocoons of* Saturnia carpini.

In the Trans. Ent. Soc. Lond., 1892, Part I., p. 45, I gave an account of some experiments touching the variation of the colour of the cocoons of the Small Egger (*Eriogaster lanestris*), and of the Emperor Moth (*Saturnia carpini*). It has been stated by Poulton* and others that the familiar variation of these cocoons, from coffee-brown to a cream-white colour, takes place in accordance with the substances to which the cocoons are attached, and the inference was suggested that this variation in colour was a protective adaptation to render the cocoons inconspicuous. The evidence which I brought forward went to show that the statement that there is any relation between the colour of these cocoons and that of the substances, to which they are attached, was founded on a mistake. In the case of *Eriogaster*, experiment showed

(1) That caterpillars left to spin in the leaves of the food-plant (hawthorn) spin *dark* cocoons.

(2) That caterpillars taken away from their food and shut up spin *light* cocoons, whether the surroundings in which they are confined are black or white.

(3) That caterpillars which of their own choice crawl into and spin in white paper placed amongst their leaves spin *dark* cocoons.

From these results it was to be concluded that the cause determining the production of light cocoons was removal from the food, or the state of annoyance incident to such removal, and that in fact the light-

* E. B. Poulton, 'Colours of Animals,' pp. 142—146.

coloured cocoon was an abnormal product resulting from unhealthy conditions.

As regards *S. carpini*, of the three points given above the second was fully established. No caterpillar which was removed and shut up spun a dark cocoon. The other two points were not fully established, for, while all the cocoons which I could find wild in the hedges were dark, few comparatively of those fed in captivity spun cocoons of full colour. Several of these, however, were attached to white paper, as in (3).

Lastly, in the case both of *Eriogaster* and *S. carpini*, there was evidence to show a strong probability that the colouring matter was derived from the contents of the alimentary canal, and that in the case of the light cocoons this substance was either evacuated, or not produced, or possibly absorbed. Two points, therefore, remained for further investigation; first, whether *S. carpini*, if in healthy circumstances, will spin dark cocoons independently of the colour of its surroundings; and secondly, the far more important question of the nature and origin of the colouring substance. To the solutions of both of these questions the evidence to be given contributes.

(1). From two batches of eggs I reared about 140 larvæ of *S. carpini*. Supposing that my larvæ had not been under good conditions last year, I resolved this year to sleeve them on a bush in the open air. On the 2nd of July, therefore, when they had made their last moult, I divided them into two lots, A and B.

A. Sixty-six larvæ were placed on a large branch of hawthorn in the Botanic Garden, and were covered with a large sleeve of white muslin. Into this sleeve I put a considerable quantity of crumpled white paper, arranging it so that the paper lay thickly amongst the leaves. In the autumn, when all had spun, I opened the sleeve, and counted the cocoons, numbering 53, the remainder having presumably escaped. Of these—

7 were spun on the white sleeve.
18 were spun in the white paper, or between it and the sleeve.
19 were spun partially attached to the white paper and partly to twigs, &c.
9 were spun on leaves or twigs, not attached to the white paper or sleeve.

With one exception all these cocoons are of the full dark colour. The exception is also a brown cocoon, but it is very thin and deficient in substance, and consequently of rather a lighter colour. It is one of the 19 named above.

B. Forty-four larvæ were enclosed in a sleeve of black muslin, and placed on another branch of the same bush. Into this sleeve I put a quantity of crumpled brown paper, of the darkest colour I could get. On opening this sleeve in September, I found 48 cocoons, namely—

2 in brown paper.
1 between paper and leaves.
4 on the black sleeve.
31 in the leaves, or massed against each other.

All these were of the full dark colour. I should say that the brown paper had become so much bleached by exposure to weather that it could scarcely be called brown.

This experiment must, I think, be considered to show conclusively that there is no relation between the colour of the cocoons of *S. carpini* and that of the substances to which they are attached. We need not therefore, in this case, consider the difficult problem whether, if such a relation did exist, it might or might not be properly considered a protective device.

(2). As to the origin of the colouring substance, I have satisfied myself that it is obtained from the contents of the alimentary canal. This conclusion is made for the following reasons:—

(*a*). The white cocoons are thin and papery, while the dark cocoons are stiff and very shiny, on the inside especially, looking as if they had been stiffened with brown size.

(*b*). In the case of some brown cocoons spun against white paper, there was a brown stain on the paper, as though a brown fluid had oozed through.

(*c*). In the case of a majority of larvæ, which, in 1891, spun white cocoons, there was evidence to show that an evacuation of the contents of the alimentary canal had taken place.

(*d*). This evacuation is, when still wet, of a reddish brown colour, of a viscous consistency, and contains small pieces of chewed leaves, and sometimes half-formed fæces.

(*e*). On opening a larva, whether young or nearly full-fed, the contents of the alimentary canal are bright green, but upon exposure to the air they turn to the red-brown colour of the evacuations seen in the breeding-cages. By washing out the contents of the alimentary canal, and filtering out the *débris* of food, a clear green filtrate was produced, which turned red-brown in the course of some minutes. There can be no doubt that this change is connected with oxidation, for it takes place more rapidly if the test-tube containing the fluid is shaken, and immediately if yellow nitric acid is added. Moreover, if the contents of the alimentary canal are placed on a glass plate, the surface soon turns in colour, while the lower part next the plate may be seen to be still green. The change from green to red therefore results from oxidation.

The actual origin of this colouring matter in the alimentary canal is not easy to determine. There are two chief possibilities; first, that the green colour is a substance (such as bile, for example) secreted by the animal; or, secondly, that it is formed from the food. The first of these is almost certainly disproved by the fact that there is no green substance in the walls of the gut, or in the tissue adjacent to it, which undergoes the change described; whereas, if the substance were the result of secretion, it would be expected that this would be the case. Filling the tissue-spaces surrounding the gut there is indeed a green fluid, but this retains its colour on exposure unchanged, not even turning to black, as do the body-cavity fluids of so many larvæ.

If, then, the colouring substance is not a secreted body, but is formed in some way by digestion from the food, the question naturally suggests itself, is it a chlorophyll product? That this is so is on the whole likely, but I know no way by which it might be proved to be so. For since the whole gut is filled with chewed leaves, there is of necessity much chlorophyll present, and it is not possible to obtain the colouring substance free from chlorophyll.

In this connexion it should be remembered that the brown colour of the cocoons is a very good match with the brown to which hawthorn leaves turn in winter, and it is not unlikely that the change from green to brown undergone by the colouring substance of the cocoons may be akin to that which takes place in the leaves. This suggestion is, of course, merely made for what it is worth.

If the contents of the gut are dried, the brown substance remains perfectly soluble in water.

(*f*). The proof that the green colouring matter from the gut is used to dye the cocoon brown rests on the following observations:—If a larva is irritated it ejects from the mouth a green glairy fluid, which turns red-brown, like the contents of the gut. If a piece of a *white* cocoon be laid in this fluid for some minutes, it soon acquires the brown colour of a brown cocoon, from which it is indistinguishable. The same is true of a fibre of silk drawn from a spinning animal, which can be dyed in the same way. The colour is then insoluble, and cannot be washed out, having stained the silk like a mordant. In the previous experiments, in 1891, I was puzzled by finding the colour soluble in the evacuations, but insoluble in the cocoons; but this is no doubt the explanation.

(*g*). Lastly, it is to be considered how the colour gets from the animal's gut to the silk. As to this, I have no decisive evidence. I know that a caterpillar may spin brown threads without touching them with the posterior end of the body, and it is therefore clear that the colour may be given out from the mouth, just as it is when the larva is irritated. But the appearance of the interior of a cocoon rather suggests that a large quantity of the size-like matter has been poured out at once. It seems possible, therefore, that there may be a final discharge from the intestine after the cocoon is finished. I am inclined to think that some of the threads are often spun white, and smeared with the colour afterwards, for I have seen threads of a cocoon lately begun, first white and then brown after an absence of an hour, and the animal may often be seen, as it were, "mouthing" over its threads. This is not always the case, for I have seen very dark threads lying adherent to the surface of paper, in such a position that they could not have been gone over again without staining the paper, but must have been put down brown while still viscous.

I think, then, it may be safely concluded (1), that the brown colour of the cocoons is derived from the alimentary canal; (2), that it is produced in the digestion of the food, and that it is *probably* a chlorophyll-derivative; (3), that it is imparted to the silk from the mouth of the larva, and perhaps by evacuation from the intestine also.

I have to thank Dr. A. Sheridan Lea for kindly advising me in the examination of this substance.

II. *The colours of pupæ of* Vanessa urticæ.

The pupæ of *V. urticæ* and of some other butterflies are known to be sometimes much pigmented, and sometimes very light, with little or no pigment. Apart from the pigmentation, they also vary greatly in the extent and brightness of the metallic lustre, which is so marked a feature of these forms. Poulton* has described experiments showing that there is a relation between these variations and the colours of the linings of the cages in which the larvæ pupated. In the past summer I made experiments of the same kind on *V. urticæ*, and the results fully bore out Poulton's account, to which I can add little.

The larvæ were collected when about half-grown, and were put into shallow cardboard boxes, through one end of which the stem of a nettle was passed. The boxes were lined with one or other of the following papers:—(1) gilt, (2) silver, (3) yellow, (4) white, (5) black, or (6) painted with Indian ink. The face of each box was covered with a pane of glass, and the boxes were all placed upright in a row facing a south window. During the three weeks through which the experiments continued there was generally a bright sun, so that the boxes became very hot. In some of them there was a good ventilation maintained, while others were kept very close, so that by the transpiration from the plant the atmosphere of the box was saturated with moisture, which also trickled continually down the glass. I did not find that the condition of moisture or dryness affected the colours of the pupæ. It is perhaps unnecessary that these experiments should be described in detail, as Poulton's description is complete.

One series of experiments, made by way of control, have, however, some interest, as materially confirming the view that the change in the colours is really due to

* E. B. Poulton, Phil. Trans., 1887, vol. clxxviii., B, p. 311.

the action of light. A number of larvæ were shut in gilt boxes as described, and these were immediately placed in a dark, closed cupboard, which was not opened again until the larvæ had pupated. With few exceptions all these pupæ belonged to the darkest class (see table). Other larvæ were put in a black box and similarly treated, with the same result.

The larvæ were collected from various places round Cambridge, and belonged to some dozen or more batches of larvæ, but I distributed the families among the boxes so as to test the existence of any congenital differences as regards pupal colour, but found none.

Some interest attaches to the fact that the great proportion of larvæ collected by me were infested with *Tachinæ*. Probably, in round numbers, five or six larvæ died from *Tachina* for one that pupated, but those that did pupate almost without exception emerged. There is therefore no reason to suppose that either the gilt pupæ or the dark ones are diseased.

	Much Pigment.			Some Pigment.			Little or no Pigment.		
Amount of Gilding.	None.	Some.	Much.	None.	Some.	Much.	None.	Some.	Much.
Gold paper ..		2		2	5	9		9	14
Silver do. ..				2	2			2	3
White do. ..				4				2	2
Yellow do. ..					1				5
Black do. ..	9	5			1	1			
Indian Ink ..	10	6	1	2	3		1	2	
Shut in the dark:—									
Gold	26	3			1				
Black	9								

Taken together—gold, silver, yellow, and white papers gave 2 dark, 16 moderate, 23 light; black paper and Indian ink gave 31 dark, 5 moderate, 3 light; gilt paper, shut in the dark, gave 29 dark, 1 moderate; black paper, shut in the dark, gave 9 dark.

There are, of course, two things to be thought of: first, the pigmentation; secondly, the metallic colours. As the table shows, both these qualities seem to be affected by the surroundings. As Poulton has mentioned, the metallic appearance is an interference-colour, disappearing when the pupæ are dried, returning when they are wetted. Of the physiology of these phenomena I have gleaned no hint at all.

The whole question touching the putative utility of these colours as a protection, seems to me an unprofitable field for study. As to the enemies of these creatures, other than insect-parasites, there is almost no evidence, and as to the senses by means of which these parasites seek their prey, there is still less. Of enemies to any of these forms in the pupal state, there is, so far as I know, no direct evidence at all. The pupal state is very short, lasting about a fortnight or three weeks, according to the weather, and the view that these peculiar colours have been developed by these creatures to conceal them from imaginary enemies during that brief time is, in my judgment, quite unsupported by fact. This view is applied to the case of these pupæ by an indiscriminate extension of deductions made in other cases fairly enough, as, for example, in that of the larvæ of *A. betularia* (*v. infra*).

After experience of these pupæ, the doubt whether the metallic colour can in any way lead to their concealment is stronger than it was. The gilded pupæ, so far as I can see, do not by reason of their gilding approximate to the appearance of any natural substance, either of flakes of mica, or to the dried slime left by slugs, or to any other bright objects to which they have been compared by ingenious persons. If Mr. Poulton had spoken of this gilding as a "warning coloration," I should have been less surprised.

One thing more may be said. In the case of the sole, in the case of the larvæ of *A. betularia*, and the like, there can be no doubt that the change of colour represents an "attempt" on the part of the animal to approximate to the colour of its surroundings. Now, in the case of these gilt pupæ, do we really know that the change represents any such effort at approximation? I confess that, though as regards the deposit of pigment this may be so regarded, the change in the degree of metallic colouring does not seem to me to be an approximation of this kind at all. It is true that gilt paper makes some approach to the look of these pupæ, but the yellow paper, and silver or white papers, do not in the least. In this connexion a circumstance, which I saw several times, may be mentioned. In several cases a larva in a gilt box pupated, not on the gilt paper, but on leaves of the food-plant, so that it was not anywhere exposed to the paper; sometimes, indeed, when practically surrounded by a leaf or leaves, and among these were some of the most golden pupæ. Notwithstanding, therefore, the clear evidence that the proximity of brightly illuminated surfaces promotes the production of the metallic appearance in these pupæ, I cannot see that there is any reason to suppose that this is a "protective resemblance," or, indeed, that it is a "resemblance" at all.

In his work on this subject, Mr. Poulton, indeed, admits that by reason of their metallic lustre the pupæ do not resemble any substance to which they are attached in nature; but he suggests that perhaps they may have come through a phylogenetic phase in which they did attach themselves to such substances. Though nothing forbids anyone from framing such an hypothesis, it is surely evident that if conjectures of this kind are to be admitted as a basis for argument, all zoological science will be thrown into confusion.

III. *The colours of larvæ of* Amphidasys betularia (*the Pepper Moth*).

Mr. Poulton was kind enough to send me some newly-hatched larvæ of *A. betularia*, with the suggestion that I should repeat his interesting experiment described in 'The Colours of Animals,' 1890, pp. 152 and 153. Larvæ reared among green leaves and green twigs only, were said to be green through life, while larvæ reared on leaves amongst which darkly coloured sticks were placed were stated to assume a dark colour.

My experiment has substantially verified Mr. Poulton's account. When the larvæ came to me they were of a kind of medium brownish green colour, being rather more brown than green. They were divided into four lots on the 12th of July.

Two lots (A) were fed on green leaves (*Populus nigra*) without black sticks, and two lots (B) were fed on green leaves amongst which black sticks were placed. Care was taken that the leaves given to all were from shoots of similar age.

It is scarcely necessary to describe the course of the experiment in detail, as Mr. Poulton has already done so; but I may give the conditions seen at two examinations:—

24th July. *Lot A.* Originally 13. Of these 8 were of the full bright green colour, 2 were brown-green, and 2 were brown.

Lot B. Originally 14. Of these 12 were very dark in colour, 1 was green, and 1 was dead.

I then took all the sticks out from among the B lot, and put them with Lot A. On the 7th of August the result was as follows:—

Lot A. 7 very green, 2 medium brownish green, 1 darker, but not of the full dark colour; 2 dead.

Lot B. 12 still very dark, 1 green as before.

No further change in colour took place, so far as I could judge. The effect therefore, once produced, seems not to be reversible, as it is in the case of the sole and the like. The change of colour is, as Poulton says, produced by the deposit of dark pigment in the one set of larvæ, and by the absence of it in the other.

It should be mentioned that these larvæ, like many other *Geometræ*, are almost exclusively night-feeders, and rarely move by day. Those provided with black sticks sat *either* on them or on the green twigs of their food throughout the day. Of course, in this case the resemblance to sticks in the one case and to green twigs in the other is unquestionable, and I think it may be fairly argued that this resemblance may contribute to the protection of the animal.

My best thanks are due to Mr. Poulton for giving me an opportunity of making this experiment, which I have watched with great interest.